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CIS-ELEMENT ARCHITECTURES OF THE PROMOTER REGIONS AND EXPRESSION PATTERN OF MYO-INOSITOL OXYGENASE (MIOX) GENES IN ARABIDOPSIS THALIANA

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ABSTRACT

In-silico analysis was performed to analyse the promoters of four MIOX genes in Arabidosis thaliana. The analyses revealed that several regulatory elements were present in upstream promoter region (1200 bp from start codon) of Arabidopsis MIOX (AtMIOX) genes. Some of regulatory elements were common while the number of elements differed in AtMIOX promoter sequences. The highest expression of AtMIOX4 was noticed in flower and pistil, whereas AtMIOX1 and AtMIOX5 were highly expressed in endosperm of seed. MYB binding site (MBS) involved in drought stress were present in promoter sequence of AtMIOX2 and AtMIOX4. Expression pattern of MIOX genes positively correlated with the similar number of cis-regulatory elements and motifs presented in the promoter regions. The presence of specific cis-regulatory elements and motifs indicate that the response of MIOX is highly controlled by abiotic (heat and drought) and biotic (fungi and bacteria) stresses. The information generated by this study could pave the way for functional and crop improvement studies in economically important crop plants.

KEYWORDS: In-Silico, MIOX Genes, AtMIOX Genes, Abiotic & Biotic

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Original Article

INTRODUCTION

The transcription regulation of gene in higher eukaryotes is usually controlled by their promoter sequence. The promoter is a non-coding DNA, composed of *cis*-regulatory elements (CREs) that is positioned upstream region of gene (Berendzen et al. 2006; Wittkopp and Kalay 2011). CREs contain binding sites for transcription factors and/or other regulatory molecules which are required for initiation and regulation of transcription of gene (Aggarwal et al. 2015).The presence and absence of specific CREs in the promoter sequence define the spatio-temporal expression of a gene (Wang et al. 2009).The spatio-temporal pattern carries an essential information to understand the certain physiological role of the gene (Pan 2006; Pan et al. 2012).

Myo-inositol oxygenase (*MIOX*) gene family is known to be involved in phytic acid, cell wall and ascorbic acid biosynthesis pathways. MIOX enzyme is responsible for the oxidative conversion of *myo*-inositol into D-glucuronic acid, which is further used as precursor in various pathways (Valluru and Van den Ende 2011; Alok et al. 2015). MIOX regulates the concentration of *myo*-inositol, which plays an important role in plant growth, development and synthesis of a variety of molecules (Alok et al. 2015). *MIOX* is encoded by four conserved member of genes located on different chromosomes in *Arabidopsis thaliana* (Lorence 2004; Kanter et al. 2005; Siddique et al. 2009; Alford 2012). These genes are differentially regulated in tissues and plant

developmental stages .*Arabidosis thaliana MIOX2* (*AtMIOX2*) predominantly expressed in all tissues whereas, *AtMIOX4* and *AtMIOX5* were expressed only reproductive stages (Kanter et al. 2005). The expression of *AtMIOX2* and *AtMIOX4* were suppressed in shoot but not in root by exogenous exposure of glucose. These two genes were expressed during low energy/nutrient conditions (Alford 2012). MIOX role has been characterized in soybean and rice (during abiotic stress) and Arabidopsis (during biotic stress)(Siddique et al. 2009; Siddique et al. 2014; Höller et al. 2015; Chen et al. 2015). Exogenous application of *myo*-inositol positively correlated with the expression of *MIOX* in root and leave tissues of wheat(Alok et al. 2015).

The regulation of *MIOX* gene family is complex and still not correlated with CREs present within their promoter regions. Hence, the identification and *in-silico* characterization of CREs in *AtMIOX* promoters could be a great interest to understand their role in *MIOX* expression. In the present study, we have analyzed the CREs of *AtMIOX* promoters and correlated them with the expression profiles by using available microarray database. The present study might be useful to implement in complex crops such as wheat to produce stress tolerant transgenic varieties.

MATERIAL AND METHODS

Genes Structure Analysis and Isolation of 1.2kb Upstream Region of Genes

The known nucleotide sequences of all four members of *AtMIOX* (*AtMIOX1*, MNM_001160865.1; *AtMIOX2*, NM_127538.4; *AtMIOX4*, NM_118759.5 and *AtMIOX5*, NM_125047.3) were searched in Ensembl Plants (http://plants.ensembl.org/index.html) against Arabidopsis using BLAST program. FGENESH+ program was used for the prediction of exon and intron in the genomic contig (www.softberry.com). The genomic sequence with 1200 bp upstream of 5'-terminus of each cDNA were selected and exported in FASTA format.

In-Silico Analysis of Cres of MIOX Promoters

The identification of various CREs in the promoters of *MIOX* was analyzed by using PlantCARE (http://bioinformatics.psb002Eugent.be/webtools/plantcare/html/) database using 1200 bp upstream gene regions. All possible CREs known in plant system were searched by using PLACE database (http://www.dna.affrc.go.jp/PLACE/). All CREs were mapped from -1 to -1200 bp upstream gene region using Regulatory Sequence Analysis Tools (RSAT) (http://www.rsat.eu/).

In-Silico Analysis of MIOX Promoter for CpGIslands

The 1200 bp upstream region of each *AtMIOX* was analyzed for the presence of CpG islands by using CpG Finder (www.softberry.com) and CpG island search database (http://dbcat.cgm.ntu.edu.tw/). The parameters for identification of CpG in minimal length 200 bp were set as GC content 50 %, observed/expected CpG as 0.60.

Microarray Expression Analysis of *AtMIOX* Gene Family

The probes ID for all four *AtMIOX* genes were searched from PlexDB an array database. The spatio-temporal and tissue specific expression patterns of these genes werestudied using their respective probe set IDs. The expression of these four genes was analyzed across various samples representing tissue specific, plant developmental stages, heat, light, drought, elicitors and biotic stress conditions. They were clustered into heat maps to compare expression of candidate genes using GENEVESTIGATOR software (Zimmermann et al. 2004).

RESULTS AND DISCUSSIONS

Gene Structure and CpG Islands of *AtMIOX*

Genomic information of Arabidopsis through Ensembl Plants and FGENESH+ program revealed that *AtMIOX2*, *AtMIOX4* and *AtMIOX5* contain 11exons whereas *AtMIOX1* has only 8 exons (Figure 1). The CpG islands was absent in sequences of all *AtMIOX* promoters. In wheat, *MIOX* contains nine exons in each homolog (Alok et al. 2015). This shows that the genomic structure of *MIOX* is varies within species and inter-species.

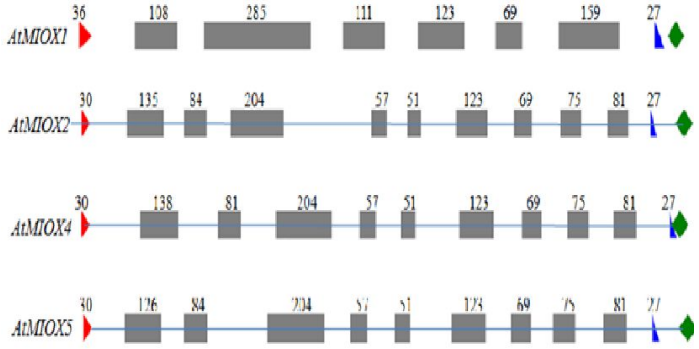

Figure 1: Genomic Structure of *MIOX* Gene Family in Arabidopsis Thaliana. Gray Boxes and Blue Line Indicate Position of Exons and Introns, Respectively. Nucleotide Length of Exons is shown Above of Box. Red, Dark Blue and Green Colors Indicate the Positions of Start Codon, Stop Codon and Poly a Tail, Respectively

Analysis of the Regulatory Cis-Elements

In order to elucidate the mechanism of transcriptional regulation of *AtMIOX*, analysis of their promoter region for the *cis*-elements was performed. A 1200 bp sequence upstream from start codon was subjected in PLANT CARE for promoter analysis, which showed the presence of multiple *cis*-elements for each of *AtMIOX* gene. For more clarity, the CREs mapped by RSAT to their location in the upstream gene region between -1 to -1200 bp (Figure 2).

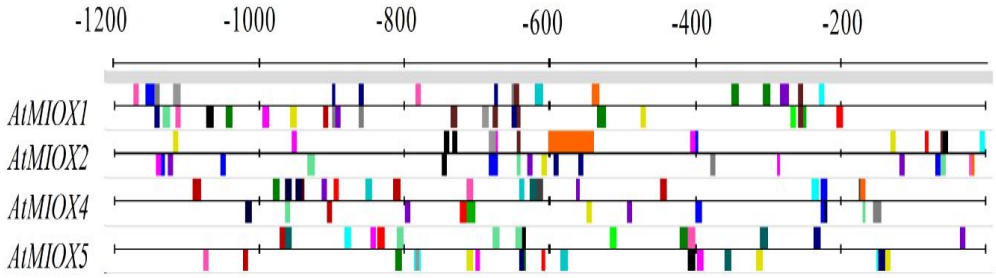

Figure 2: Cis-Regulatory Elements (Cres) Map in the Promoters of *Atmiox* (-1 To 1200 Pb). The Similar Colour on the Different *Atmiox* Indicates the Position of a Particular Type of *Cis*-Element

Promoter sequences of *AtMIOX* have different CREs which is listed in Table 1. These CREs mainly belong to six classes as; elicitor specific, hormone responsive elements, light responsiveness, binding site specific, stress-responsive elements and plant tissues specific.

Table 1: List of Different CREs in Promoter Sequences of all Four *AtMIOX* Genes

Promoter	CREs and Motif Found Promoter Sequences
AtMIOX1	AAACmotif, AAGAAmotif, ABRE, ARE, AT rich element, ATGCAAAmotif , Box 4, CGTCA motif, G Box, GAmotif, GAREmotif, GT1motif, Gap box, HSE, LTR , TC-rich repeats, TCA-element and TGA-element
AtMIOX2	ACII, ARE, ATCTmotif, Box 4, BoxW1, CATTmotif , GBox, GAmotif, GAGmotif, GAREmotif, GATAmotif, GCN4motif , I box, MBS,Skn 1 motif, Sp1, TGACGmotif, W box and circadian
AtMIOX4	3-AF1 binding site, AAGAAmotif, ABRE, ACE, AEbox, ARE, Box 4, BoxW1, CGTCAmotif, GBox, GAREmotif, GATA-motif, GCN4motif, HSE, I-box, LAMPelement, MBS, RY-element, Skn-1motif , TC-rich repeats, TGAelement, TGACG-motif, W box, WUNmotif, chs-CMA2b, circadian and rbcS-CMA7a
AtMIOX5	3-AF1 binding site, AAGAAmotif, ACE, ARE, Box I, Box III, BoxW1, CATbox, CCAATbox , ERE, Gbox, GAmotif, GAGmotif, GAREmotif, GCN4motif, GT1motif, Gapbox, HSE, LAMPelement, MBSII, MSAlike, Pbox, Skn1motif, Sp1, TC-rich repeats, TCTmotif, W box, as2box, box E, box II and circadian

Expression Patterns of *AtMIOX* in Tissue Specific and Plant Developmental Stages

The differential expression patterns of all *AtMIOX* were noticed in different tissues and plant development stages (Figures 3A and B). The heat map showed the expression of *AtMIOX2* was ubiquitous in almost all tissues and plant development stages (Figures 3A and B). The highest expression of *AtMIOX4* was found in flower and pistil, whereas *AtMIOX1* and *AtMIOX5* were highly expressed in endosperm of seed. The presence of Skn1 motif in promoter of *AtMIOX5* was strongly suggested its expression in endosperm. We did not found endosperm expression specific CREs in *AtMIOX1* promoter.

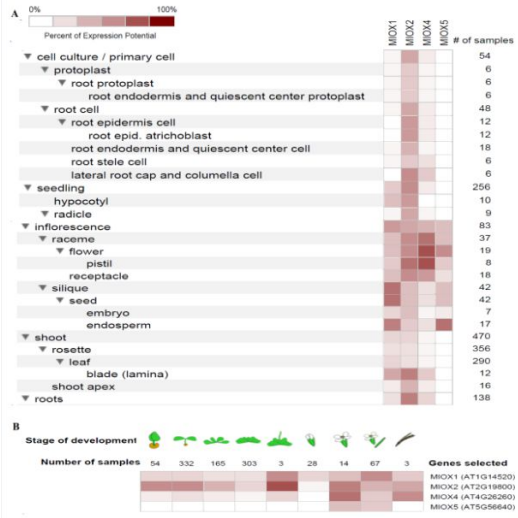

Figure 3: Microarray Based Expression Profiles of *Atmiox. (*A) Tissue Specific Expression, and (B) Expression during Plant Developmental Stages. The Heat Map Was Generated Using Meta-Analysis Tool at GENEVESTIGATOR (https://www.Genevestigator.com/gv/plant.jsp). Colours Represent the Fold Change for Gene Expression

Expression Profiles of *AtMIOX* during Abiotic Stresses

To correlate the expression pattern of *AtMIOX* genes with CREs in their promoter under abiotic stress conditions, we have generated two heat maps using available GENEVESTIGATOR database (Figures 4A and B). The one group of heat map included the expression response due to abscisic acid (ABA), blue light and heat (Figure 4A), whereas other

group showed the expression under drought, light/dark, methyl jasmonate (MeJa) and salisylic acid (SA) (Figure 4B). The expression of *AtMIOX1* was highest in seedling and whole plant sample during the ABA stress condition. The presence of ABA responsible element (ABRE) at four different locations in *AtMIOX1* promoter is strongly correlated with the high expression of it in these tissues (Figure 4A). ABRE was absent in *AtMIOX2* and *AtMIOX5* promoters. Real-time PCR analysis in rice showed the expression of *MIOX* was stress inducible by drought, salt, and ABA treatments (Duan et al. 2012). The heat stress responsive CRE is present in *AtMIOX1* and *AtMIOX4*, however the heat map showed positive correlation with *AtMIOX1* expression (Figure 4A). MYB binding site (MBS) are responsible for drought stress and located in *AtMIOX2* and *AtMIOX4* promoters and it is absent in remaining other two MIOX promoters (Table 1). *Glycine soja MIOX1* showed its role in alkaline stress(Chen et al. 2015). The expression of *AtMIOX2* slightly upregulated in case of cell suspension and seedling due to MeJA treatment, whereas *AtMIOX2* and *AtMIOX4* were highly upregulated in seedling during SA treatment.

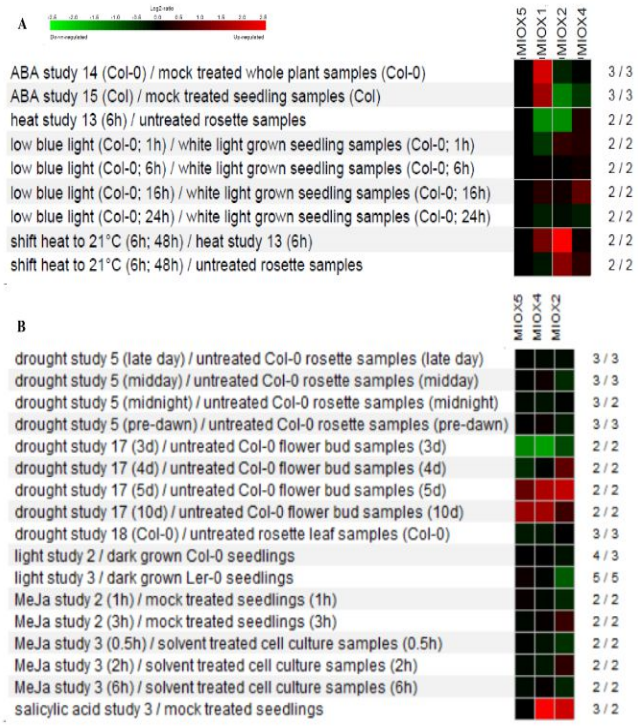

Figure 4: Microarray Based Expression Profiles of Atmiox during Different Abiotic Stresses and Elicitors. (A) Expression Due to ABA, Low Blue Light and Heat (B) Expression Due to Drought, Light/Dark, Meja and SA. The Heat Map Were Generated by GENEVESTIGATOR Tool and Grouped into Two Parts Due to Available Probeset ID for Specific Sample. Colours Represent the Fold Change for Expression

Expression Profile Analysis of *AtMIOX* during Biotic Stresses

To correlate the expression pattern of *AtMIOX* under biotic stress conditions, *Colletotrichum tofieldiae*, and *Colletotrichum incanum* (fungi), and *Pseudomonas syringae* (bacterium) were included for the study by using GENEVESTIGATOR database. *C.Tofieldiae* transfers the phosphorus (an important macronutrient) to shoots, promotes growth of plant, and increases fertility only under phosphorus deficient conditions (Hiruma et al. 2016).

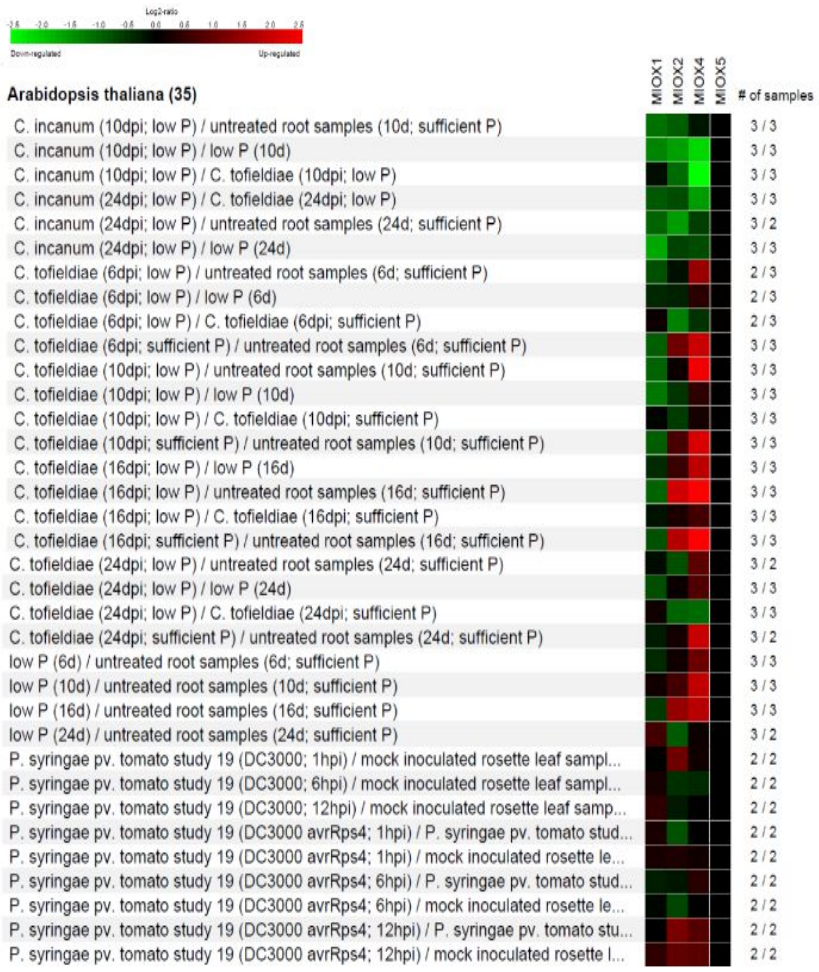

Figure 5: Microarray Based Expression Profiles of *Atmioxs* during Biotic Stresses. The Heat Map Was Generated by Genevestigator Tool and Colours Represent the Fold Change for Expression

The expression of *AtMIOX4* was highly down regulated in root due to combined biotic stresses of *C. tofieldiae* and *C. Incanum* (Figure 5). Low phosphorus condition was upregulated the expression of *AtMIOX4* and in some extent it also increased *AtMIOX2* expression. Alone stress governed by *C. Tofieldiae* in low or sufficient phosphorus conditions is highly upregulated the expression of both *AtMIOX4* and *AtMIOX2* genes (Figure 5). *AtMIOX2* and *AtMIOX4* expression was slightly upregulated due to *P.syringae* stress. Fungal elicitor responsive element (Box W1) is present in *AtMIOX2* promoter sequence, whereas defence and stress responsive (TC rich repeats), wound responsive element (WUN motif) andfungal elicitor responsive element (Box W1) were found in promoter sequence of *AtMIOX4*. Response to nematode infection in Arabidopsis showed strong upregulated expression of *AtMIOX4* and *AtMIOX5* in roots (Siddique et al. 2009).

CONCLUSIONS

The MIOX is a key enzyme of *myo*-inositol oxidation pathway in plants. The product D-glucuronic acid is an important precursor of various biosynthetic pathways. The expression of *MIOX* in plant is varies with tissue and organ specific manner. Variation noted in the upstream promoter regions of the *MIOX* in Arabidopsis is responsible for the differences in the tissues specific expression levels. The CREs analysis indicated that the transcriptional regulation of *MIOX* is controlled by abiotic (heat and drought) and biotic (fungi and bacteria) stresses. The present study could be the

step forward in the direction to identify candidate MIOX gene and promoter for the development of genetically improved and stress tolerant variety of plants.

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